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ASSOCIATION OF CYCLOPOID COPEPODS WITH THE HABITAT OF THE MALARIA VECTOR *ANOPHELES AQUASALIS* IN THE PENINSULA OF PARIA, VENEZUELA

EVELYN ZOPPI DE ROA,[1] ELIZABETH GORDON,[1] EDIE MONTIEL,[1] LAURA DELGADO,[1] JESÚS BERTI[2] AND SANTIAGO RAMOS[1]

ABSTRACT. The southern region of the Paria Peninsula shows a high malaria incidence. This work relates the abundances of cyclopoid species and the malaria vector *Anopheles aquasalis* to certain abiotic parameters and vegetation features. Samples were collected over a 4-month period in several habitats, including marsh, irrigation channel, lagoon, and mangrove swamp during the wet season and the wet–dry transition. Dominant plant species in the marsh were *Typha dominguensis* and *Eleocharis mutata. Mesocyclops meridianus* also was dominant in the marsh. Highest densities of *An. aquasalis* larvae, as well as lowest pH values and highest sulfate concentrations, were found in habitats containing *E. mutata.* Statistical correlation analysis showed that abundances of *M. longisetus longisetus* and *An. aquasalis* larvae were positively and significantly correlated in the irrigation channel, and abundances of *M. meridianus* and *An. aquasalis* larvae were negatively and significantly correlated in the *E. mutata* marsh.

KEY WORDS Copepoda, Cyclopoida, *Anopheles aquasalis,* wetlands, biological control

INTRODUCTION

The peninsula of Paria, in northeastern Venezuela, was accountable during the last 2 years for 42% of all malaria cases reported for Sucre State, the country's region of highest malaria prevalence. Investigation in Paria Peninsula of a variety of wetlands and mosquito breeding sites calls for an examination of key ecological interactions of all vector stages, especially immatures. Results of these examinations may ultimately lead to development of biological control programs. As part of a longer-term study, we intended to determine basic natural relationships between mosquito larvae and ubiquitous zooplanktonic organisms, and the importance of common abiotic and biotic environmental variables.

Cyclopoid copepods have been identified in the past decades as predators of mosquito larvae. Hurlburt (1938) suggested that the cyclopoid *Microcyclops varicans* (Sars) could be a predator of 1st-stage larvae of mosquitoes. Bonnet and Mukaida (1957), Suárez et al. (1984), Marten et al. (1989), Brown et al. (1991), Marten et al. (1994), Schreiber et al. (1996), Rawlins et al. (1997), and Santos and Andrade (1997) reported predation on larvae of various mosquito species, and recognized cyclopoid copepods as important regulators of mosquito populations, including malaria vectors. These cyclopoid predators included *Mesocyclops aspericornis* (Daday), *M. venezolanus* Dussart, *M. longisetus longisetus* (Thiébaud), *M. edax* (Forbes), *M. ruttneri* Kiefer, *Megacyclops latipes* (Lowndes), *Diacyclops navus* (Herrick), *Macrocyclops albidus* (Jurine), and *Acanthocyclops vernalis* (Fischer).

Abundances of *Anopheles albimanus* Weidemann and *An. pseudopunctipennis* Theobald have been closely related to vegetation cover and physical and chemical water conditions in Chiapas, Mexico (Rejmánkova et al. 1991). We report here that the abundance of several cyclopoid species and the larval abundance of *Anopheles aquasalis* Curry are related for various types of habitats. Some spatial and temporal relationships also are discussed regarding copepod and mosquito larval abundances, and related abiotic parameters and vegetation features.

MATERIALS AND METHODS

The Paria Peninsula, located in northeastern Venezuela, Sucre State, comprises an area of 1,078 km^2. Slope varies between 0 and 5%; annual precipitation varies between 1,000 and 1,800 mm, in the eastward direction; and air temperature ranges from 25 to 27°C. The land remains wet most of the year and water accumulates in clearly differentiated zones, such as agricultural irrigation channels, lagoons, marshes, and mangrove swamps. The mangrove swamps have been disconnected from marine influences for about 50 years and marsh areas currently are managed as water buffalo farms.

Four habitats, all potential habitats for malaria vectors, were selected: Catuaro irrigation channel, Río de Agua marsh (2 localities), Bohordal lagoon, and Irapa mangrove swamp (Fig. 1). These localities were identified as anopheline developmental sites in earlier studies (Berti et al. 1993a, 1993b).

Plant and animal material was collected during the wet season and the transition period (wet–dry) from September (1998) to January (1999). A 2-stage monthly sampling was used for each habitat. Vegetation samples were taken from 10 × 10-m plots in each representative area (2, 3, or 4 samples depending on habitat type), and 3 random samples

[1] Instituto de Zoología Tropical, Facultad de Ciencias, Universidad Central de Venezuela, Apartado 47058, Caracas 1041-A, Venezuela.

[2] Escuela de Malariología y Saneamiento Ambiental, Avenida Bermúdez, Maracay, Estado Aragua, Venezuela.

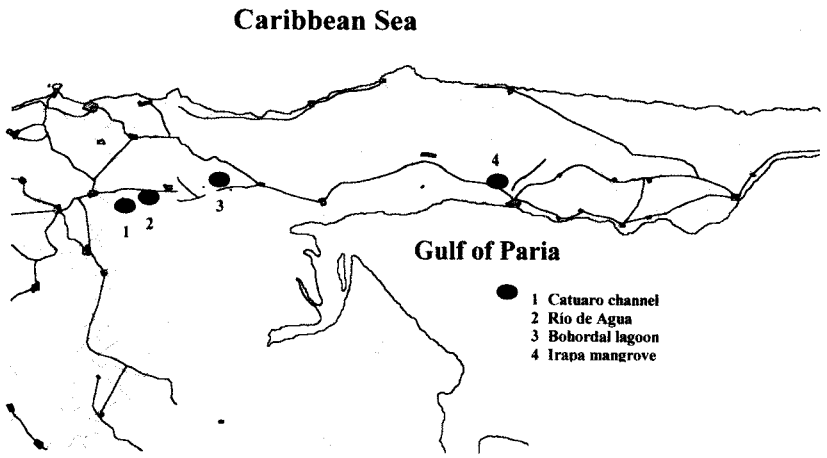

Fig. 1. Sampling sites in the peninsula of Paria, Venezuela.

were taken per site for zooplankton and anopheline larvae (Cochran 1980). Zooplankton samples were collected in a 1-liter LaMotte bottle (Chestertown, MD). Three compound samples of 10 dips each were taken with a standard mosquito dipper in each sampling site and the number of anopheline larvae was recorded for each dip for estimating mosquito larval populations. Simultaneously with zooplankton and mosquito sampling, physical and chemical variables were measured in situ with a Horiba model U-10 water quality tester (Kyoto, Japan), and water samples were collected for laboratory analysis. Plant species were identified with the aid of a taxonomic key (Velásquez and Scorza 1998). Mosquito larvae were identified with the key by Cova-García and Sutil-Oramas (1971).

Data were analyzed with the nonparametric Kruskall–Wallis test ($P \leq 0.05$), with copepod species, mosquito larvae, and physical and chemical water parameters as the dependent variables, and locality, season, and vegetation type as the independent variables. The nonparametric Spearman correlation test ($P \leq 0.05$) was performed to determine any relationship between copepod species and larvae of *An. aquasalis*. (Connover 1980).

RESULTS

Dominant vegetation and abiotic parameters associated with different habitat types are shown in Table 1. Bohordal lagoon presented steep banks with water depth from 0.99 to 2.5 m. The free-water area was dominated by submersed–floating *Utricularia inflata* Walter and free-floating *Wolfia brasiliensis* Wedd. A few isolated individuals of floating-leaved *Nymphaea ampla* (Salib) D.C. and free-floating *Azolla filiculoides* Lam were also found. Helophytes, including *Hymenachne amplexicaulis* Rudge., *Acrostichum aureum* L., *Acroceras zizanoides* H.B.K., and *Sacciolepis striata* (L.) Nash, were found along the shore. This lagoon is surrounded by arboreal vegetation more than 20 m tall.

Two vegetation types were found in the Río de Agua marsh, 1 dominated by *Typha dominguensis* Pers. and the other by *Eleocharis mutata* (L.) Roem. and Schult. In the former, water depth varied between 0.27 and 0.43 m., and vegetation cover of *T. dominguensis* varied between 70 and 80%. Other species recorded in the *T. dominguensis* marsh were free-floating *Lemna* sp., submersed *Utricularia gibba* L., and the helophytes *E. mutata* and *Cyperus articulatus* L. In the *E. mutata* marsh, water depth is managed mechanically for activities associated with raising water buffalo. Average water depth was 0.50 m. *Eleocharis mutata* cover varied between 25 and 30%. Additionally, isolated individuals of *Nymphaea* sp. were found in this marsh.

Depth in the Catuaro irrigation channel varied between 1.2 and 1.25 m. Plant species composition ranged along the channel from areas with 100% cover of *Eichhornia crassipes* (Mart.) Solms to areas with 100% cover of *Pistia stratiotes* L.

The Irapa mangrove swamp had an average water depth of 0.55 m. Vegetation was almost exclusively *Avicennia germinans* L., which patchily covered up to 60% of the swamp surface.

During the 4-month sampling period, the lowest values of water pH and highest salinity and sulfate concentrations were recorded in the *E. mutata* marsh. Bohordal lagoon showed the lowest values for concentrations of sodium, chlorides, and sulfates. All localities showed no significant variation in these variables throughout the sampling period (Table 1), except for the Irapa mangrove swamp. The Kruskall–Wallis test applied to vegetation types showed differences in the variables chlorine and sodium between the mangrove swamp and the marshes (*T. dominguensis* and *E. mutata*). Significant differences in salinity were found between the

Table 1. Vegetation and mean habitat values for each locality studied in the peninsula of Paria.

Locality	Season	Habitat	Plant species	Cover (%)	Na^+ (mg/liter)	Cl^- (mg/liter)	SO^{4+} (mg/liter)	pH	Salinity (‰)
Bohordal	Sept. 1998	Lagoon	*Utricularia inflata*	35	5.3	10.1	1.25	5.6	0.02
			Wolfia brasiliensis	25					
Catuaro		Channel	*Eichhornia crassipes*	100	—	107	34	6.38	0
Río de Agua		Marsh	*Typha dominguensis*	80	143	225	312	8	1
Río de Agua		Marsh	*Eleocharis mutata*	25	199	236	1,293	3.1	3
Irapa		Mangrove	*Avicennia germinans*	60	618	950	209	7	2
Bohordal	Oct. 1998	Lagoon	*U. inflata*	40	6.4	9.60	1.15	6.1	0.01
			W. brasiliensis	35					
Catuaro		Channel	*E. crassipes*	100	34.8	55	66	6.8	0
Río de Agua		Marsh	*T. dominguensis*	70	59.5	112	23	6.9	0.4
Río de Agua		Marsh	*E. mutata*	30	146	330	712	2.9	1.2
Irapa		Mangrove	*A. germinans*	60	438	1,431	182	7	1.5
Bohordal	Dec. 1998	Lagoon	*U. inflata*	50	5.24	10.70	1.30	7.2	0
			W. brasiliensis	45					
Catuaro		Channel	*E. crassipes*	100	44.3	46	33	7.3	0.02
Río de Agua		Marsh	*T. dominguensis*	70	85.2	176	144	8	0.04
Río de Agua		Marsh	*E. mutata*	30	178	330	706	3.2	1.2
Irapa		Mangrove	*A. germinans*	60	1,047	3,583	392	8	1
Bohordal	Jan. 1999	Lagoon	*U. inflata*	30	5.4	9.30	1.18	8.4	0
			W. brasiliensis	50					
Catuaro		Channel	*E. crassipes*	100	51.8	47	36	7.2	0
Río de Agua		Marsh	*T. dominguensis*	75	65.6	71	43	9.0	0.03
Río de Agua		Marsh	*E. mutata*	30	188	333	702	3.2	1.3
Irapa		Mangrove	*A. germinans*	60	1,902	2,890	434	8.9	1.3

mangrove swamp and the *E. mutata* marsh ($P \leq 0.05$), but not among other sites.

Eleven species of copepods were found, including 9 cyclopoids, 1 calanoid, and 1 harpacticoid. *Mesocyclops meridianus* (Kiefer) and *Ectocyclops herbsti* (Dussart) were found in all sites, followed by *Halicyclops exiguus* (Kiefer). *Metacyclops* sp. was found only in Bohordal lagoon and the Irapa mangrove swamp. The richest habitat for copepods (9 spp.) was the Catuaro channel and the poorest (2 spp.) was the *E. mutata* marsh (Table 2).

Mesocyclops meridianus was the most abundant species in all sites. In general, during December (wet–dry transition period), *M. meridianus* reached its highest density in the mangrove swamp, whereas *M. longisetus longisetus* reached its highest density in the Catuaro channel. The greatest density of *Microcyclops ceibaensis* (Marsh) in the Catuaro chan-

Table 2. Copepod species present in each locality.[1]

Copepods	Bohordal lagoon	Catuaro irrigation channel	Río de Agua		Irapa mangrove swamp
			Typha marsh	*Eleocharis* marsh	
Nauplii	+	+	+	+	+
Copepodids	+	+	+	+	+
Adults					
Ectocyclops herbsti (Dussart)	+	+	+	+	+
Ectocyclops rubescens (Brady)	+	−	−	−	+
Halicyclops exiguus (Kiefer)	+	+	+	−	+
Metacyclops sp.	+	−	−	−	+
Mesocyclops meridianus (Kiefer)	+	+	+	+	+
Mesocyclops longisetus longisetus (Thiébaud)	+	+	+	−	−
Microcyclops ceibaensis (Marsh)	+	+	−	−	−
Microcyclops anceps anceps (Richard)	−	+	+	−	−
Acanthocyclops sp.	−	+	+	−	−
Prionodiaptomus colombiensis (Thiébaud)	−	+	−	−	+
Harpacticoids	−	+	+	−	−
Number of species	7	9	7	2	6

[1] +, present; −, absent.

Table 3. Mean density of principal copepod species and *Anopheles aquasalis* larvae (individuals/liter).

Species	Month	Bohordal lagoon	Catuaro channel	Río de Agua		Irapa mangrove
				Typha	*Eleocharis*	
Ectocyclops herbsti	September	0	14	2	0	0
Ectocyclops rubescens		0	0	2	0	0
Mesocyclops meridianus		0	12	92	0	0
Mesocyclops longisetus longisetus		0	3	1	0	0
Microcyclops ceibaensis		0	6	0	0	0
Anopheles aquasalis		0	2	1	15	1
E. herbsti	October	0	9	0	0	2
E. rubescens		0	0	0	0	2
M. meridianus		0	14	13	0	8
M. longisetus longisetus		0	3	0	0	0
M. ceibaensis		0	258	0	0	0
A. aquasalis		0	1	3	4	3
E. herbsti	December	0	3	0	0	0
E. rubescens		0	0	0	0	0
M. meridianus		34	12	47	0	163
M. longisetus longisetus		0	37	26	0	0
M. ceibaensis		0	48	0	0	0
A. aquasalis		0	8	1	4	0
E. herbsti	January	5	14	11	2	0
E. rubescens		1	0	0	0	0
M. meridianus		2	13	28	4	0
M. longisetus longisetus		1	1	4	0	0
M. ceibaensis		1	0	0	0	0
A. aquasalis		0	1	1	15	14

nel was recorded in October. *Ectocyclops herbsti* was most abundant in the Catuaro channel in September and January.

Larvae of *An. aquasalis* were absent in Bohordal lagoon, and were most abundant in the *E. mutata* marsh. Mosquito larvae were more abundant in September (wet season) and January (wet–dry transition) (Table 3).

Significant differences in densities of *E. herbsti, Metacyclops* sp., *M. ceibaensis,* and *An. aquasalis* between localities were noted with the Kruskall–Wallis test. Differences were found in densities of *E. herbsti* and *M. ceibaensis* in the Catuaro channel, and *Metacyclops* sp. in Bohordal lagoon. Densities of *An. aquasalis* were different between *T. dominguensis* marsh, *E. mutata* marsh, and the mangrove swamp. No significant differences were found in copepod and mosquito larval densities among the study months; however, *M. longisetus longisetus,* which was most abundant only in December, obviously showed a significant difference for the month factor (December).

Mesocyclops meridianus showed a significant negative correlation with *An. aquasalis* ($r = -0.97$, $P \leq 0.05$) in the *E. mutata* marsh. At the Catuaro channel, *M. longisetus longisetus* was positively and significantly correlated with *An. aquasalis* ($r = 0.82$, $P \leq 0.05$), and *Ectocyclops rubescens* (Brady) was negatively correlated with vector larval density ($r = -0.68$, $P \leq 0.05$). The other copepod species did not show significant correlation with the malaria vector.

DISCUSSION

Correlation between copepod species and *An. aquasalis* varied according to vegetation type characteristic of each habitat. *Anopheles aquasalis* was absent in Bohordal lagoon. The lagoon's vegetation was mainly Lemnaceae and *U. inflata* and the lagoon was surrounded by trees more than 20 m tall. Areas dominated by Lemnaceae have been considered to be unsuitable breeding places for mosquitoes (Rejmánkova et al. 1991). Likewise, Marten et al. (1996) found that sites that were shaded by trees at the edge of water or were covered completely with floating plants, such as duckweed, were least favored for oviposition by *An. albimanus.* Highest density of *An. aquasalis* larvae in this study was in the *E. mutata* marsh, a locality with the lowest copepod richness and density, lowest vegetation cover, high exposure to sunlight, and low salinity. Marten et al. (1996) found that a site's exposure to the sun as well as its salinity seem to be important in the distribution and abundance of *An. albimanus.*

Santos and Andrade (1997) found significant correlation between a dengue vector mosquito species (*Aedes albopictus* Skuse) and *M. longisetus longisetus* and, to a lesser extent, *M. albidus* var. *albidus,* both of which are predators on mosquito larvae. Marten et al. (1989) found a strong negative association between large cyclopoids and larvae and pupae of *An. albimanus.* In their study, large cyclopoids including *M. longisetus longisetus* were found to be the most effective predators. Unlike the

findings of Marten et al. (1996), in this study we found a positive relationship between *M. longisetus longisetus* and *An. aquasalis* in irrigation channels, which might indicate that this copepod has no predatory preference for *An. aquasalis,* and may consume other organisms. However, our results showed that *M. meridianus,* a smaller-sized species than *M. longisetus longisetus,* was negatively correlated with *An. aquasalis,* suggesting that a relationship may depend on habitat type. Negative correlations cannot be interpreted as a direct predator–prey relationship, because they could result from low density of 1 of the populations. Identification of abiotic and biotic variables that might influence the dynamics of such populations is necessary. In this respect, Marten et al. (1989) argued that negative associations could point to the role of other environmental factors. According to presence–absence data for *An. aquasalis* and *M. meridianus,* Pernía (2000) found that *M. meridianus* preys on 1st- and 2nd-stage larvae of *An. aquasalis,* confirming their predator-prey relationship. This is the 1st time that *M. meridianus* has been reported to be a predator of larvae of *An. aquasalis* in Venezuela.

A prey–predator relationship has been recognized for the 1st time between anopheline and several cyclopoid species under natural conditions in Venezuelan wetlands. Densities of larvae of *An. aquasalis* and copepods depended on plant habitat type, as well as copepod species composition and abundance. These important relationships between copepod species and environmental conditions should be investigated further. Determination of positive and negative relationships between zooplankton (e.g., copepods) and mosquito larvae lends support to hypotheses dealing with the feasibility of natural control of malaria vectors in rural environments. Interdisciplinary and systemic approaches may prove valuable in solving malaria or epidemiologic and other public health problems.

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